PHARMACOKINETICS OF NIACINAMIDE IN BLOOD AND SKIN OF HAIRLESS GUINEA PIGS

Bongiovanni, R., Koplovitz, I., Schulz, S., Lieb, J., Railer, R., Mayer, T., and Shutz, M.

Drug Assessment Division
United States Army Medical Research Institute of Chemical Defense
Aberdeen Proving Ground, Maryland 21010-5425

ABSTRACT

Niacinamide (NA) has been reported to be effective in reducing the development of microblisters caused by sulfur mustard (HD) vapor exposure in the hairless guinea pig when given as a single bolus pretreatment 30 min prior to HD vapor exposure (Yourick et al.). The purpose of these experiments was to establish the pharmacokinetics of NA in the hairless guinea pig to optimize the evaluation of NA against HD cutaneous injury. A high performance liquid chromatography (HPLC) method was developed for the quantitation of NA in blood and skin. The method was linear (corr coeff r = 0.998) and sensitive with a working range from 50 μg/ml to 2000 μg/ml. The NA $T_{1/2}$ was measured after a bolus injection of 750 and 375 mg/kg via IP and IV routes, respectively. The $T_{1/2}$ was 2.8 ± 0.3 hr for both routes. Drug concentrations in blood, during multiple dosing (5 IP) of a fixed dose (375 mg/kg, i.p.) given every 2.8 hr, were within 15% of the theoretical values calculated using a computer model (Principle of Superposition). NA serum levels ranged from 325 μg/mL to 1404 μg/mL (n = 12). The corresponding skin levels were within 93% of the blood levels. The elimination of NA from the skin paralleled its elimination from the blood. The results of these studies will aid in the future evaluation of NA as a pretreatment/treatment for HD injury.

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INTRODUCTION

Niacin and niacinamide (NA) are important for regulation of the nucleotides NAD^+ and $NADP^+$. These nucleotides are coenzymes for numerous dehydrogenases and are involved in hydrogen ion transport, ATP formation and in glycolysis[1]. As a member of the vitamin B complex, niacin also plays a role in human nutrition. An inadequate dietary supply of niacin (normally about 10 mg per day) and/or its precursor tryptophan[2] leads to pellagra in man and to similar deficiency syndromes in animals[3]. Niacin also exerts a number of pharmacological activities at doses higher than the normal daily requirement of this vitamin[4]. Clinical applications of niacin include treatment of pellagra and chronic alcoholism[5], as well as as a hypolipedemic agent[6].

Unfortunately, niacin has been shown to elicit undesirable side effects. At moderately high levels, niacin treatment (100-150 mg/day) causes a variety of side effects including vasodilation, altered blood flow, fibrinolytic activation and at higher doses (1-6 g/day) hypolipemic actions[3,7,8]. In contrast, NA has been used to treat pellagra in humans without the side effects associated with medium to high dose niacin treatment[3]. In addition, NA has an immediate and rapid effect. Patients who experience cutaneous pain or soreness due to inflammation or ulceration caused by necrobiosis lipoidica have reported improvement after the initiation of high-dose niacinamide (NA) treatment, and recession to pre-treatment level upon cessation of therapy[9].

Another potential cutaneous application of NA is as a pretreatment for chemical blistering agents. NA was shown to provide partial protection in in-vivo animal exposures to sulfur mustard (2,2'-dichlorodiethylsulfide; HD)[10]. In a subsequent study, pretreatment and multiple treatment doses of NA (750mg/kg) administered to Hairless Guinea Pigs (HGPs) led to a significant decrease in epidermal-dermal microblister formation 72 hours after exposure to HD, as compared to HD exposed animals given as single pretreatment dose of NA or saline injections of the same volume[11].

The present pharmacokinetic (PK) animal model for NA described here was developed and the data further utilized to establish varying plasma NA steady-state levels (C_{ss}). These C_{ss} levels were further utilized to assess drug efficacy to HD exposure levels. A High Performance Liquid Chromatography (HPLC) method was developed for the measurement of NA in blood. It is simple, requires a minimum amount of sample (20-50 μL), minimum amount of time for sample preparation, and a specific chromatographic separation. It is ideally suited for a large number of low volume samples encountered in PK experiments.

EXPERIMENTAL

Animal Use and Care

Male [Crl:IAF/HA(hr/hr)BR Vaf/Plus] euthymic hairless guinea pigs (Cavia porcellus), 250-650g, from the Newfield, NJ breeding facility were used. Upon arrival, they were quarantined and screened for evidence of disease before use.

They were maintained under an AAALAC accredited animal care and use program. Guinea pigs were individually housed in plastic cages (Lab Products, Inc., Maywood, NJ) on shredded corn cob bedding (Bed O'Cobs, The Andersons, Industrial Products Division, Maumee, Ohio/Delphi, ID, U.S.A.) which was changed three times per week. Commercial certified guinea pig ration (Ziegler Bros., Inc., Gardners, PA, U.S.A.), and tap water were given ad libitum. Animal holding rooms were maintained at 21°C ± 2°C with 50% ± 10% relative humidity using at least 10 complete air changes per hour of 100% conditioned fresh air. All guinea pig cages were covered with filter paper (Remay #2016, Research Equipment Co., Inc., Bryan, TX, U.S.A.) to minimize heat loss. Animals were maintained on a 12 hour light/dark full spectrum light cycle with no twilight.

For this study, HGPs were given a single IP or IV (via vena cava) injection of NA at 750 or 375 mg/Kg, respectively. Blood was drawn from the animals at specific time points after bolus administration. All blood draws were taken from the vena cava using 1cc tuberculin syringes (Becton-Dickinson (BD) Rutherford, NJ U.S.A.) with 26 gauge, 1/2 inch needles (BD). The plasma was separated by centrifugation at 3900 rpm for 15 minutes using microtainer blood sample separation vials (BD). Tubes were kept on ice before, during, and after blood collection. Plasma was stored at -10°C until analysis.

Materials

NA, isoniacinamide (isoNA, internal standard), monobasic sodium phosphate, and dibasic sodium phosphate were all obtained from Sigma (St. Louis, MO, U.S.A.), while 200 proof anhydrous ethanol was purchased from Warner-Graham (Cockysville, MD, U.S.A.). HPLC grade acetonitrile was purchased from J.T. Baker Inc (Phillipsburg, NJ, U.S.A.). Water was collected from a Millipore (Milford, MA, U.S.A.) Milli-Q UF PLUS water purification system.

HPLC Equipment included an LC-6A chromatograph equipped with an SIC-6B auto injector, SCL-6B system controller, SPD-6A UV Detector (D_2 lamp 254 nm detection wavelength, AUFS 0.01), and C-R4A Chromatopac integrator was used (All parts from Shimadzu, Kyoto, Japan). The samples were chromatographed on a reversed phase PRP column (Hamilton) at ambient temperature. The mobile phase was aqueous buffer (pH 6.0, 0.20 M sodium phosphate)/acetonitrile (90:10, v/v), which was delivered at a rate of 1 mL/min. All mobile phases were filtered and degassed under vacuum with a 0.65 micron membrane (Millipore, Milford, MA, U.S.A.). Other equipment used included the following: a refrigerated centrifuge (Beckman Accuspin FR, Fullerton, CA, U.S.A.), and a small tabletop centrifuge (Dynac, Parsippany, NJ, U.S.A.), a vortex samole mixer (Cybron, Dubuque, Iowa, U.S.A.).

Extraction and Analysis

Into a 1 ml plastic centrifuge tube was placed 20 μL of serum, 20 μL of the internal standard isoNA (250 μg/mL), and 160 μL of cold (-20°C) ethanol which had been kept on dry ice. The mixture was vortexed for 30 seconds and centrifuged at 3500 rpm for 20 minutes. Routinely, a 10μL sample was analized by HFLC.

Pharmacokinetics Analysis

Pharmacokinetic parameter estimates V_{ss} = apparent volume of distribution at steady state (mL/Kg), AUC = area under the plasma concentration-time curve (μg mL^{-1}hr), K01-HL = rate constant of absorption half life (hrs^{-1}), α-HL = rate constant of elimination half life (hrs^{-1}), β-HL = overall rate of elimination half life (hrs^{-1}), A & B = concentrations at time 0 (μg/mL), C_{max} = maximum concentration (μg/mL) , and T_{max} = maximum time (hrs) were determined by PCNONLIN (Statistical Consultants, Inc., Version 4.0, 1993) non-linear regression analysis. Each time-concentration profile was characterized in terms of the parameter estimates that produced the best fit to the standard model. The mean parameter estimates were calculated and are reported as the mean ± standard error of the mean. The statistically calculated concentrations were used for graphical representations of time vs concentration utilizing SIGMA PLOT Version 5.0 (Jandel Scientific, San Rafael, CA).

RESULTS

Calibration, recovery, linearity and sensitivity

The present method was used to evaluate the recovery efficiency and linearity of NA. Standard aqueous solutions (50 μg/mL to 2000 μg/mL) of NA were used to determine a linearity range for the present study. The biological sample standards used were prepared as outlined in the extraction and analysis section. **Figure 1** illustrates sample chromatographic analysis of aqueous (panel A) and extracted serum samples (panel B), each spiked with 200 μg/mL of NA standard.

Sample recovery was calculated by comparing the concentration of the drug in serum to a linear standard curve. It was established by preparing a series of spiked samples, aliquoted, and frozen (quality control). These were routinely analyzed with the unknown samples. During a period of two months, there was no apparent degradation of the frozen samples and the linear regression slopes to include the areas of standard / internal standard ratios were within a 92% or better agreement between data sets.

The recovery of NA in plasma was >95% when compared to an aqueous standard curve. This is to be expected, because the procedure is simply deproteinizing the serum proteins (serum samples) with a dilution factor of 1:10 (see extraction procedure volumes). Routinely, an excellent linear range was achieved between 50 μg/mL to 2 mg/mL. The lowest workable concentration was 5 μg/mL. This was a practical range for the pharmacokinetic studies of NA. **Figure 2** represents the recovery of a representative NA (50 μg/mL to 5 mg/mL) study. Recovery was defined by the following linear regression equation with a correlation coefficient (r); Y = 1.43 X + 0.00, r = 0.998 (aqueous): Y = 1.55 X + 0.002, r= 0.998 (spiked plasma). For this study, the recovery of NA in serum was an average of 108.4 %. Routinely, prior to the pharmacokinetic animal preparation studies, blood samples were collected from normal HGPs and analyzed for NA. The range was 39 to 111 μg/mL (n=12) endogenous levels of NA.

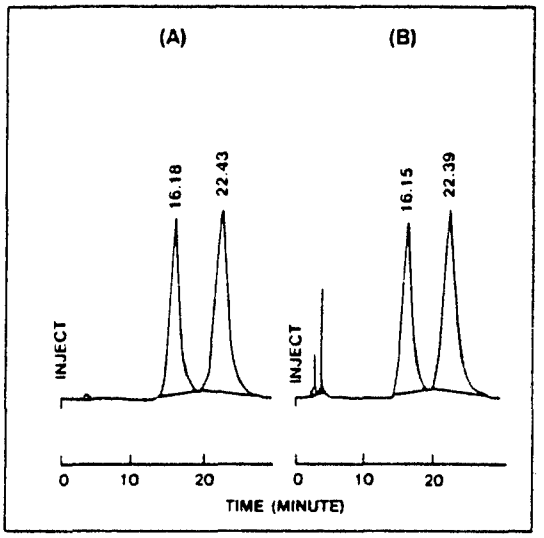

Figure 1. HPLC chromatographic analysis of an aqueous sample (panel A) and a spiked serum sample (panel B), containing niacinamide (200 μg/mL) and the internal standard isoniacinamide (isoNA, 250 μg/mL). Panel A retention time of niacinamide 22.43 min. and isoniacinamide 16.18 min., AUFS 0.01, attenuation 0 and 15 μL injection on column.

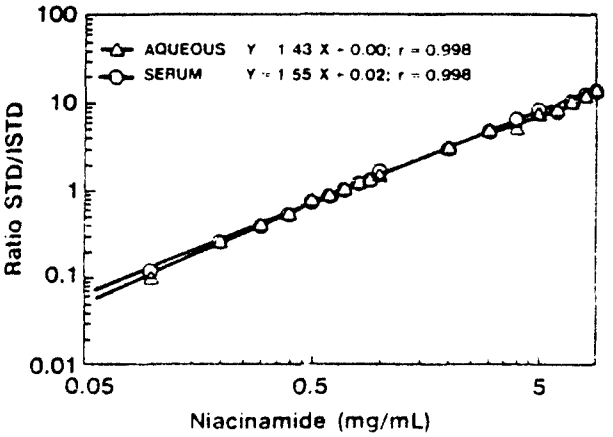

Figure 2. Recovery study of spiked biological serum samples (o) compared to a theoretical curve of NA (Δ) standards (50 μg/mL - 5 mg/mL) injected on column.

Validation and application

Independent studies were conducted for the PK of niacinamide by IP and IV administration. Routinely, a group of 3 to 5 HGPs at approximately 2 months of age with a selected mean body weight not to exceed a 2% variation between animals were chosen for the disposition kinetics. Each animal was given an IP or IV bolus adjusted volume (75 mg/mL, NA in distilled water) to reflect a 750 and 375 mg/Kg, respectively. The total volume was delivered within 1.0 minute. An average of three blood samples were collected from each animal with a time interval of 2-3 hrs. Blood samples were collected at specific time intervals (see Figures 3 - 5 for specific time points). The samples were placed on ice immediately and the plasma removed within 30-45 minutes. Plasma samples were stored frozen until the time of analysis. These were analyzed and the results statistically treated as described in the methods section.

The plasma level at steady state was validated as follows: Six animals were IP administered NA (375 mg/Kg) at 2.8 hrs (T1/2) intervals (5 IPs). After 5 IP administrations, samples were routinely drawn 10 min. post last administration and analyzed. The plasma levels were 731.3 ± 21.71 (mean ± s.e.m.) and were within a 10 % agreement, when compared to a computer model (principle of superimposition)[19].

DISCUSSION

There have been a wide variety of techniques employed for the purpose of determining the plasma and urine concentrations of nicotinic acid and its derivatives. Early assays utilized microbiological response comparisons[12], which cover only a small concentration range and cannot resolve niacin and NA. More recently, paper chromatography and thin layer chromatography assays were used but these are time consuming with considerable loss of analyte[13]. Colorometric assays are not specific, require a large amount of sample, and at times a long development time[14], and quite often nonspecific[13]. Accurate, reliable, sensitive, selective and specific assays employing fluorimetric methods[15], gas chromatography / mass spectrometry (GC/MS) analysis[16] and high performance liquid chromatography (HPLC) have been previously described[7,14,17].

The present analytical method is a single step procedure to determine NA and niacin and requires a minimum amount of sample (20 - 50 μL), followed by chromatographic analysis. To extend the level of sensitivity, the method was modified by utilizing disposable pre-column concentrators. The exact method and applications have been submitted for publication elsewhere.[18] This modified method features a linear range of detection from 500 μg/mL to 500 ng/mL with a limit of detection of 2 ng on-column injection. It also includes a method for the analysis of niacin, NA, and 1-methylniacinamide in plasma, and skin. Skin levels compared favorably (>90% agreement) to concomitant plasma level determinations. Figure 3 shows the correlation between skin (expressed in μg per gram of wet tissue) and plasma (μg/mL) concentrations over time. Note that the plasma and skin levels were within excellent agreement, except for the data set at 2 hrs post 12th IP. For this data set, both the blood and skin sample results are in agreement except that the data does not correlate well with the linear

elimination curve. We contribute the discrepancy to animal variation and not the analytical method.

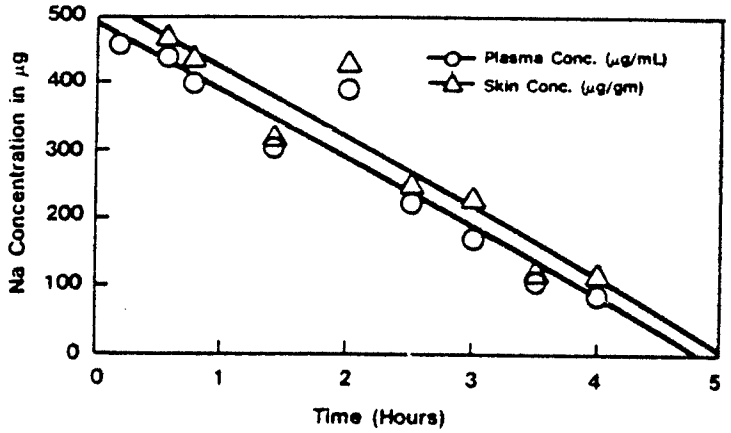

Figure 3. The correlation between skin (ug/mL, Δ) and plasma (ug/mL, O) concentrations after the twelfth IP NA (375 mg/Kg bolus dose) treatment (data points reflect actual data).

Plasma concentration-time data was found (IP studies) to best fit to an exponential equation representing a one-compartment model with first-order absorption and elimination. For the IV studies, the data was best fitted to an exponential equation representing a two-compartment model with first-order adsorption and elimination.

The model-predicted pharmacokinetic curves of two independent studies (IP, NA 750 mg/mL)) are shown in Figure 4. The actual parameters have been tabulated in Table 1. The elimination rate constant (β-HL) for the central compartment was in excellent agreement (2.78, 3.01, and 3.05 hrs.). The data was in agreement with another model (IV, NA 375 mg/mL) as shown in Figure 5 and Table 2. For the IV study , the β-HL was 2.398 ± 0.79 hrs. The overall NA half life (IP and IV) was averaged as 2.8 ± 0.3 hrs.

It has been observed that NA metabolism varies significantly between species[3,4,6,8,12,17,18,9]. Therefore, if a new species, such as the HGP, is used as an animal model to study niacin or NA, it is essential to characterize the pharmacokinetic parameters to ensure the validity of the model within the context of the experiment. The present study has clearly defined the parameters required to calculate Css. Finally, skin data results and the ability to accurately determine Css will aid in the future evaluation of NA as a pretreatment for HD injury.

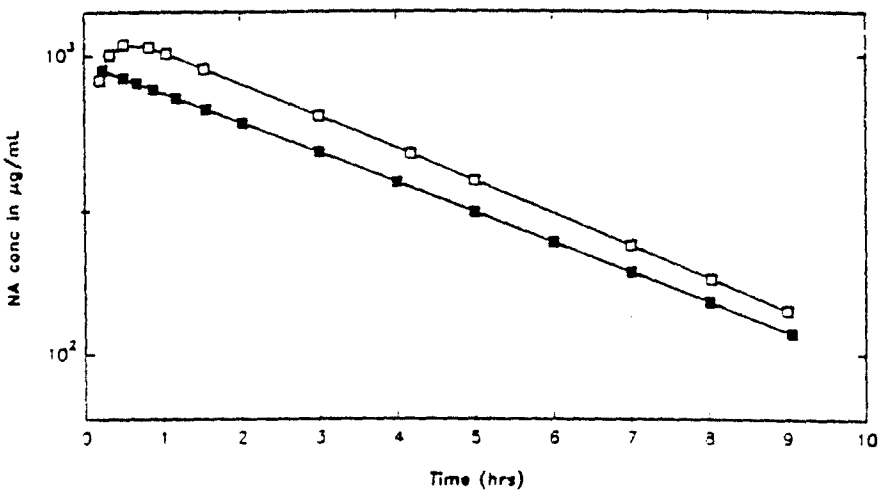

Figure 4. Model-predicted pharmacokinetic curves of two independent studies, 5 HGPs (□) and 3 HGPs (■), respectively. Each group was administered (IP) 750 mg/Kg NA. The plasma time-concentration curves reflect a one-compartment open model.

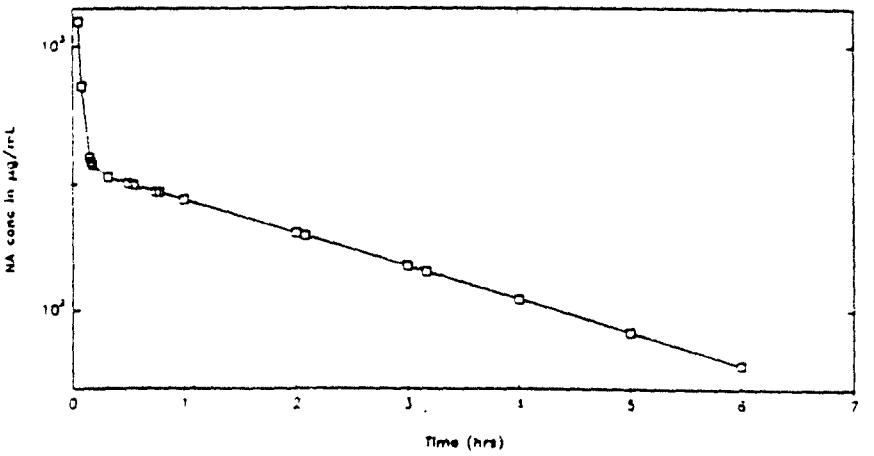

Figure 5. Model-predicted pharmacokinetic curve of NA. A group of 7 HGPs was administered (IV) 375 mg/Kg NA. The plasma time-concentration curve reflects a two-compartment system.

Table 1. Comparison of the pharmacokinetic parameters for NA using three independent studies (IP bolus administration of 750 mg/Kg)

Parameter One-Compartment model	Study I mean (± s.e.m)	Study II mean ± s.e.m	Study III mean ± s.e.m
AUC(μgmL^{-1} hr.)	5076 (583)	4071 (293)	4111 (378)
K01-HL (hr^{-1})	0.124 (0.03)	0.230 (0.15)	0.272 (0.06)
K10-HL(hr^{-1})	2.78 (0.44)	3.01 (0.18)	3.05 (0.45)
T_{max} (hrs)	0.58 (0.10)	0.17 (0.87)	1.04 (0.14)
C_{max} (μgmL^{-1})	1095 (55.20)	902 (216)	738 (321)

Table 2. Comparison of the pharmacokinetics parameters for NA (IV bolus administration of 375 mg/Kg).

Parameter Two-compartment model	Study I mean (± s.e.m.)
AUC (μg/mL^{-1} hr)	1346.9 (317.2)
α (hr^{-1})	30.5 (8.55)
β (hr^{-1})	0.289 (0.09)
α - HL (hr^{-1})	0.023 (0.006)
β - HL (hr^{-1})	2.398 (0.79)
A (μg/mL^{-1})	4125.6 (1834.6)
B (μg/mL^{-1})	350.1 (45.2)
V_{ss} (L Kg^{-1})	0.868 (0.14)

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